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# Impulse activity of locus coeruleus neurons in awake rats and monkeys is a function of sensory stimulation and arousal

(norepinephrine/sleep/neurotransmitters/electrophysiology)

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**ABSTRACT** **By means of extracellular recordings, individual norepinephrine-containing neurons in the locus coeruleus of unanesthetized behaviorally responsive rats and squirrel monkeys were found to respond to specific sensory and behavioral conditions. In rats, distinct clusters of action potentials followed the presentation of various nonnoxious auditory, visual, or somatosensory stimuli at latencies of 15–60 msec. Increased discharge rates were also seen during periods of spontaneous electroencephalogram arousal in both species. In monkeys, these cells responded most vigorously to complex arousing stimuli such as a preferred food. Because the noradrenergic innervation of most forebrain regions arises from the locus coeruleus, these results allow prediction of situations under which this massive projection system would be active and suggest a physiological role for this chemically identified network in specific behavioral processes.**

Norepinephrine (NE)-containing neurons in the brain nucleus locus coeruleus (LC) have been hypothesized to serve many functions. The hypothesis that has been most intensively tested with lesion, pharmacological, and electrophysiological techniques is that these neurons play a central role in initiating and maintaining one or more of the stages of the sleep–wake cycle (1–4). Previous studies (5–7) of LC neuronal discharge activity during the sleep–wake cycle have been consistent with this view: In cats, a subpopulation of LC neurons exhibits changes in mean discharge rate that consistently anticipate, and are characteristic of, the various stages of the cycle. However, because the cat LC is composed of interdigitated NE and non-NE neurons (8, 9), it is not possible to attribute this relationship with sleep stages specifically to the NE-neuron subpopulation; the importance of this neurochemical heterogeneity is emphasized by the very heterogeneous discharge properties of different cat LC neurons during the sleep–wake cycle (5–7). In order to surmount this problem, rats and squirrel monkeys were chosen as the experimental subjects for the present study. There is substantial evidence for both species that the LC is composed entirely of NE neurons (10, 11); thus, recordings from within the LC in these species can be assumed to arise from such neurons.

Studies on anesthetized or decerebrated animals have revealed that in these preparations LC neurons respond reliably only to noxious stimuli or to electrical stimulation of peripheral nerves (12–14); these reports have supported other hypotheses that LC is primarily concerned with fear, anxiety, and nociception (15–17). Although German and Fetz (18) have reported that monkey LC neurons exhibit no systematic changes in discharge rate during operant responding for food, and Chu and Bloom (5) have reported that cat LC neurons exhibit higher mean discharge rates during "active" waking than during "quiet" waking, there has as yet been no systematic study of LC neuronal activity and its sensory response patterns within the waking undrugged state. The present report presents evidence that, during waking, positively identified NE-containing LC neurons homogeneously exhibit pronounced responses to nonnoxious, physiological, sensory stimuli of many modalities. Furthermore, these neurons show similarly predictable alterations in discharge rates as a function of sleep stage and of fluctuations in arousal level within the waking state.

## MATERIALS AND METHODS

**Subjects.** Data were obtained from 7 adult (750–1050 g) male Guyanan squirrel monkeys and from 12 adult (350–450 g) male Wistar rats.

**Recording Techniques.** The techniques used for single-cell recording were similar for the two species (see also refs. 19 and 20). While each animal was under general anesthesia, a stainless-steel cylinder was aimed toward the LC and permanently attached to the skull. Reference microelectrodes, electroencephalogram (EEG) screws, eye-movement electrodes (monkeys only), a ground electrode, and electromyograph leads were also implanted. Recordings in unanesthetized minimally restrained animals began about 1 week after surgery; no drugs were administered during recording. Monkeys sat in a chair that allowed head, arm, and leg movement but prevented locomotion. Rats were free to move about a small enclosure (25 × 30 cm), restricted only by a counterbalanced flexible cable attached to their heads. Sharpened tungsten microelectrodes were advanced into LC by a remotely controlled hydraulic microdrive attached to the implanted cylinder. Only results with well-isolated unitary action potentials are reported here. The criteria for acceptable recordings were as follows: (*i*) stable amplitude at least twice the noise level, (*ii*) systematic predictable changes in amplitude with changes in electrode position, (*iii*) no potentials within the absolute refractory period, (*iv*) polarity and duration characteristic of soma rather than axonal action potentials. Tone pips (4 kHz, 25-msec duration, approximately 96 decibels against a 57-decibel background noise level), light flashes (10-$\mu$sec duration, approximately $10^6$ candelas), and brief, mild skin taps (manually applied to the rostral tail surface with a wooden applicator) were administered to rats at regular 5- to 10-sec intervals. Digital pulses, synchronized with these stimuli, were tape recorded and also fed to a computer. Action potentials were discriminated and digitized by a voltage-sensitive gate, and both analog and digital signals were recorded. Digital action potential pulses were also fed to a second computer input, permitting on- or off-line generation of peri-stimulus-time histograms. Electrolytic lesions for marking electrode depth were created at specific points during each microelectrode penetration by passing 5–10 $\mu$A

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Abbreviations: NE, norepinephrine; LC, locus coeruleus; EEG, electroencephalogram; A, awake; SWS, slow-wave sleep; D, desynchronized sleep.

of cathodal current for 10 sec. This procedure, combined with appropriate survival time, produced glial scars 100–250 μm in diameter at these points. One or two days after completion of all recordings, animals were perfused with formalin and serial 40-μm Nissl-stained sections through recording sites were prepared. Individual penetrations were identified by their relative placements: penetrations were limited in number and sufficiently separated (500 μm in monkeys) to permit unambiguous identification of each electrode track. The locations of recorded cells along a particular penetration were determined by correlating the positions of marking lesions with microdrive readings noted during the penetration (see Figs. 1 and 2).

Cortical EEG, electro-oculogram (monkeys), and electromyogram were recorded on a polygraph as well as being tape recorded concurrently with action potential data. Polygraph records were scored blind for waking and sleep stages, using conventional criteria.

## RESULTS

This report encompasses data from 45 neurons: 22 monkey and 23 rat. Each recording site was verified histologically as being within the compact portion of the LC, which contains only NE neurons in these species.

**Discharge Rates with Respect to Sleep Stages.** All rat and monkey LC neurons systematically altered their discharge rates with respect to sleep stages in much the same way as a previously described subpopulation of cat LC neurons (5–7): discharge rates were a function of whether the animal was awake (A), in slow-wave sleep (SWS), or in desynchronized sleep (D). To determine mean discharge rates for the monkey neurons, a 60-sec sample of each stage was analyzed for each cell. For the rat data, the mean sample length was 149 sec per stage per cell (range 23–424 sec). For the rat data, $n$ = 18, 15, and 5 cells for A, SWS, and D, respectively; for the monkey data, $n$ = 15 for A and SWS. Under the recording conditions of this study, monkeys exhibited little deep-SWS and no D. Mean discharge rates, ± SEM, (in Hz) were as follows. Rat: A = 2.12 ± 0.20, SWS = 0.69 ± 0.16, D = 0.02 ± 0.01 ($P < 0.005$ W vs. SWS, $P < 0.05$ SWS vs. D). Monkey: A = 2.45 ± 0.362, light SWS = 0.877 ± 0.137 ($P < 0.001$).

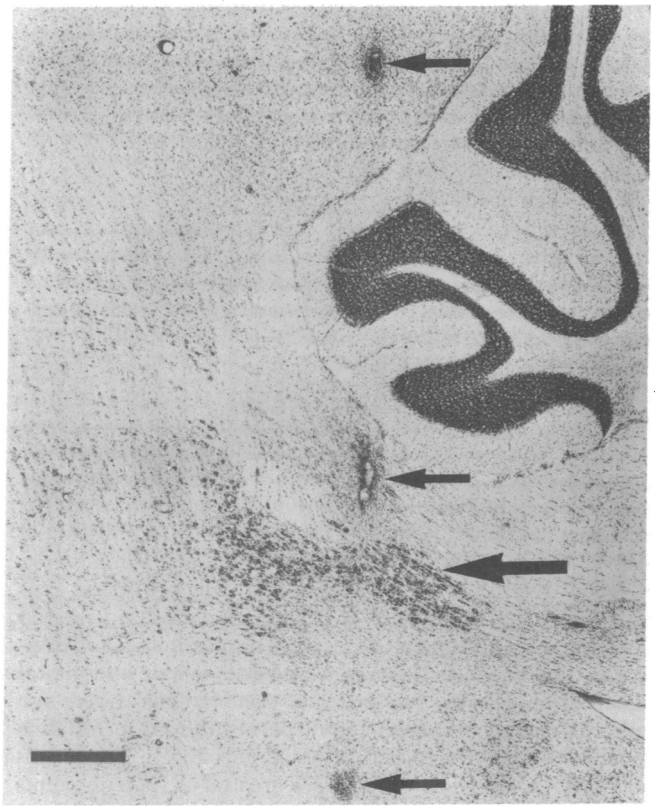

FIG. 1. Sagittal 40-μm Nissl-stained section showing microelectrode penetration through monkey LC. Gliotic scars from three marking lesions made at known depths are indicated by the small arrows. LC neurons are indicated by the large arrow. Calibration bar equals 500 μm.

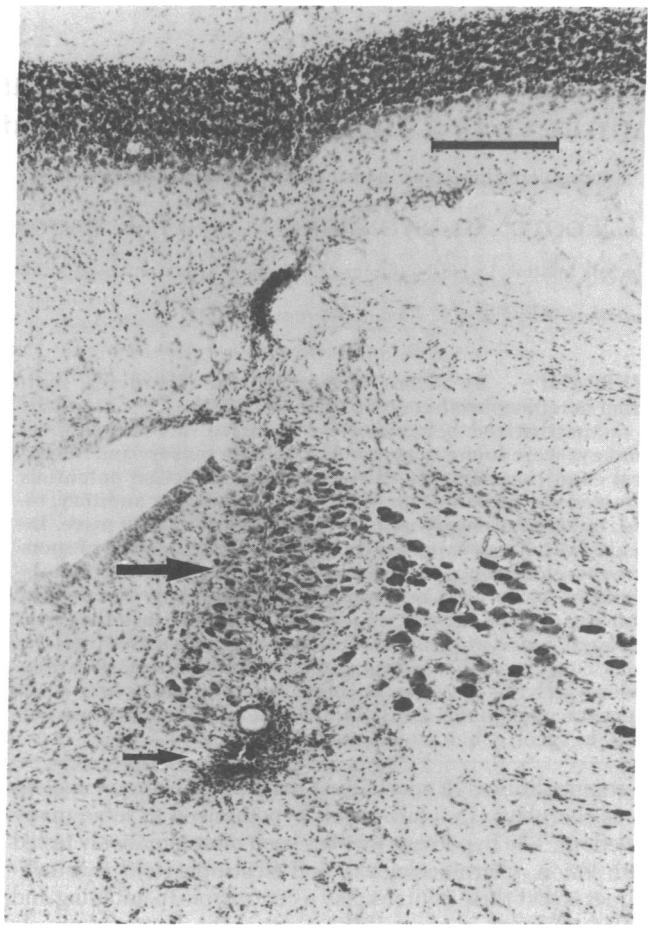

FIG. 2. Coronal 40-μm Nissl-stained section showing microelectrode penetration through rat LC. Glial scar from marking lesion made at the end of the penetration (100 μm below typical LC activity) is indicated by the small arrow. LC is indicated by the large arrow. Calibration bar equals 200 μm.

**Sensory Responses.** LC neurons in both species typically exhibited biphasic responses to nonnoxious physiological sensory stimuli in all modalities tested (i.e., visual, auditory, and somatosensory): brief increases in discharge rate were closely followed by more prolonged decreases. Table 1 catalogs the number of neurons exhibiting responses in the various sensory modalities. Fig. 3 demonstrates the polysensory responsiveness of one typical rat LC neuron. Four of the five rat neurons whose responses to all three modalities were quantitatively evaluated exhibited excitatory responses in every modality ($P < 0.05$ for light flashes and tones; doubling of rate for touch). The fifth cell exhibited excitatory responses to light flashes and touch but not to tones. Response latencies in rat were as follows (mean ± SEM): tone = 18.7 ± 1.3 msec, $n$ = 7 cells; flash = 50.0 ± 2.7 msec, $n$ = 5 cells.

Polysensory responsiveness exhibited by monkey LC neurons was more difficult to quantify because repeated presentations of the same stimulus led to rapid response diminution. Of 18

Table 1. Discharge properties of monkey and rat LC neurons

| Property | Cells exhibiting characteristic/ cells tested | |
|---|---|---|
| | Rat | Monkey |
| Total cells | 23 | 22 |
| Slow tonic spontaneous rate | 21/23 | 22/22 |
| Excitatory auditory response | 13/14 | 16/18 |
| Excitatory visual response | 8/9 | 17/19 |
| Excitatory touch response | 9/10 | 19/19 |
| Excitatory response to startle | — | 16/18 |
| Postexcitatory response decrease | 9/11 | — |
| Excitatory response to sight of food | — | 18/18 |
| Relative rates in sleep cycle: | | |
| A > SWS > D | 4/4 | — |
| A > SWS, no D | 18/18 | — |
| A > drowsy > SWS | — | 18/18 |

The minimal criterion for an excitatory response was a doubling of mean discharge rate.

monkey LC neurons tested with a variety of stimuli, 16 responded by at least doubling their firing rates to at least three consecutive repetitions of at least one stimulus in each of the three modalities. It was also observed, but not quantitatively documented, that in both species the intensity of sensory response was positively correlated with the vigor of the accompanying arousal response or orienting behavior. In the monkey particularly, when the stimulus no longer elicited EEG arousal or orienting movements, LC neurons usually no longer responded to the stimulus. LC responses were not strictly linked to any particular motor acts, however, because apparently identical nonevoked movements by the animal did not alter neuronal discharge rate.

**Arousal Responses.** In monkeys, complex arousing stimuli such as the sight of a preferred food or of an unfamiliar person in the recording room reliably increased LC activity. The highest sustained discharge rates (7–15 Hz) observed for each of 18 monkey neurons tested occurred when the monkey was shown brightly colored fruit juice in a transparent syringe from which the animal had previously been fed this preferred food (see also ref. 19). In contrast, during orientation to a novel but less arousing stimulus such as a tone, rates of 2–6 Hz were most common. During quiet waking when monkeys were behaviorally inattentive, rates of 0.5–3.0 Hz were typical. As the animals began to drowse and periods of high-amplitude EEG activity persisted for several seconds, LC discharge rates dropped to well below 1 Hz. Thus, even within unambiguous periods of waking, these cells exhibited 10-fold fluctuations in activity.

Spontaneous arousal, as indicated by decreased cortical EEG amplitude, eye movements, and increased muscle tone, was also accompanied by increased discharge rate. Fig. 4 shows a typical monkey LC neuron whose discharge rate systematically varied with fluctuations in EEG amplitude throughout both spontaneous and experimenter-elicited episodes of attentiveness or arousal. These observations were quantified by correlating the mean EEG amplitude of each 1-sec epoch with the number of action potentials occurring during that period. The mean EEG amplitude was determined by integrating the rectified EEG. Spearman's rank-order correlation coefficient was computed, using the correction for ties (21). Probabilities were computed by converting $r_s$ to a $t$ score. Each cell was analyzed for 60–400 sec (mean sample length 138 sec). For 10 monkey neurons, $\bar{x}r_s = -0.41$, $P < 0.005$ for each cell. Similarly in rat LC, discharge rates during active waking exceeded those during quiet waking.

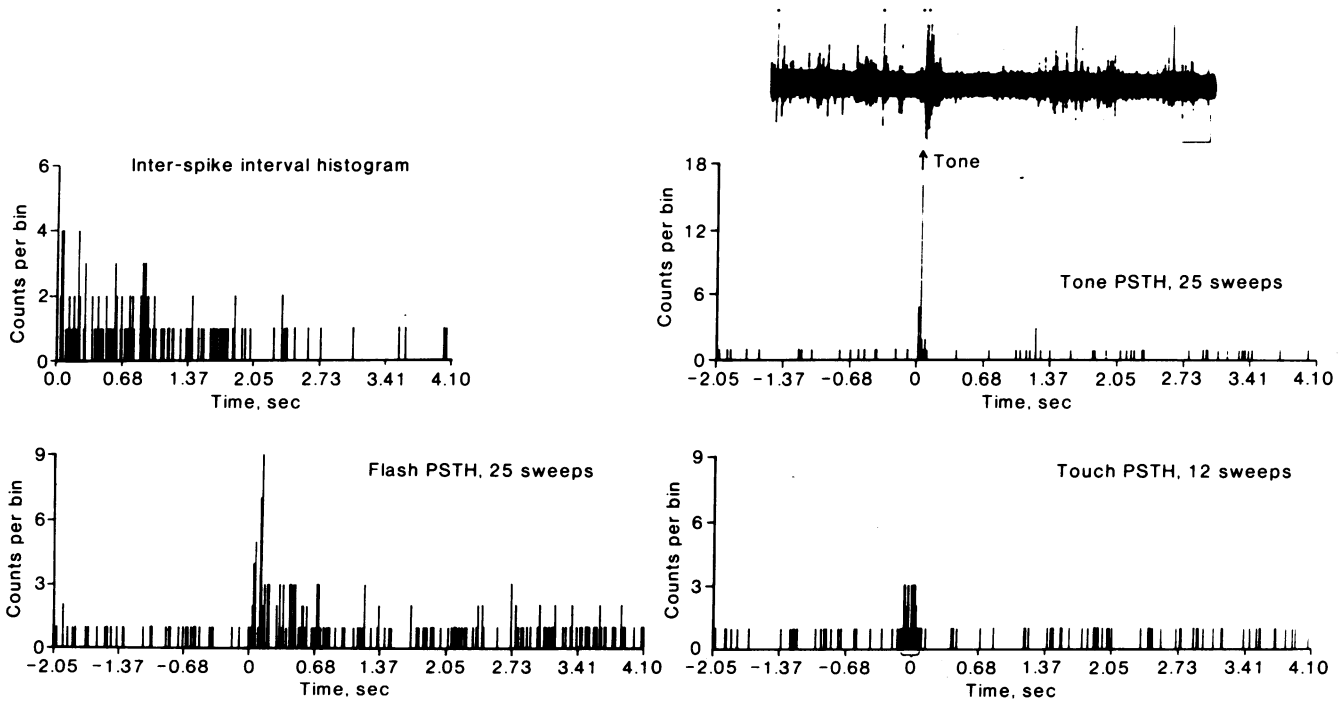


FIG. 3. Spontaneous and sensory-evoked activity of a typical individual rat LC neuron. In the inter-spike interval histogram, there were 162 total counts from a sampling period of 253 sec. In the three peri-stimulus-time histogram (PSTH) displays, the stimuli were delivered at time = 0. This neuron exhibited biphasic excitatory–inhibitory responses to stimuli in each sensory modality tested. The postexcitatory rate decrease was sometimes obscured in cumulative histograms when responses after the first 5–10 sweeps were attenuated, apparently by a combination of habituation and vigilance changes. (*Inset*) Analog record of this neuron's response to the first stimulus (arrow) of the tone PSTH display. Dots above action potentials are waveform discriminator outputs, which serve as the input to the computer. Calibration marks equal 50 $\mu$V and 400 msec.

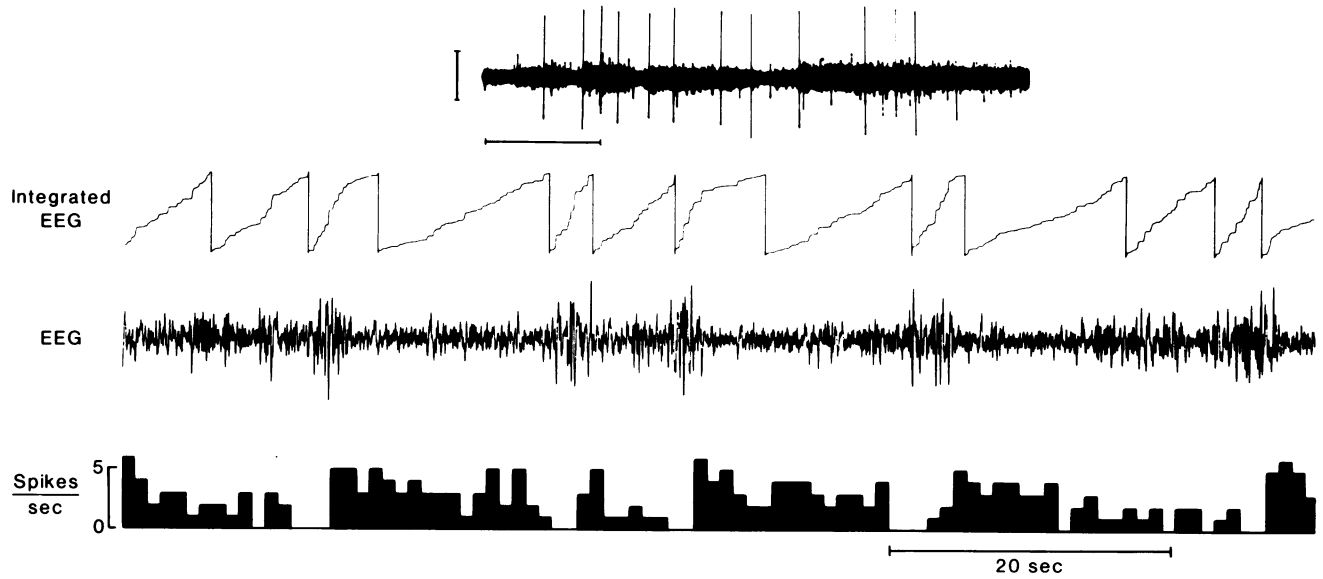


FIG. 4. Typical relationship between monkey LC neuronal activity (bottom trace) and cortical EEG during an episode of behavioral wakefulness. Oscillographic trace of the unitary action potential is shown at the top; calibration marks equal 100 $\mu$V and 1 sec. Unit activity is higher during periods of low-amplitude EEG. This observation was quantified by correlating the number of action potentials in each 1-sec epoch with the integrated EEG value (i.e., mean amplitude) for that epoch. For this cell, for a sample of 397 sec, Spearman's $r_s = -0.53$, $P < 0.001$. Changes in the activity of rat and monkey LC neurons often anticipated spontaneous EEG changes. Thus, for this cell, if the number of action potentials in a particular second was correlated with the EEG amplitude for the following second, the $r_s$ value was increased to $-0.62$, $P < 0.001$.

2.55 ± 0.22 Hz (mean ± SEM) vs. 1.71 ± 0.20 Hz, $n = 18$ cells, $P < 0.005$.

## DISCUSSION

The nature of the relationship between LC neuronal activity and sleep stages has been previously studied (4–8). Much like a previously described subpopulation of LC neurons in the cat, rat and monkey LC neurons were most active during waking, much less active during SWS, and (at least in the rat) nearly silent during D sleep. However, data obtained from cat LC cannot be used to generalize about activity in NE projections specifically because of the uncertainty of the neurochemical identity of recorded neurons. The established neurochemical identity of the neurons yielding the strikingly uniform relationships in the present report lends strong support to previous suggestions that the physiologically similar subpopulation of cat LC neurons may be those containing NE (4, 6, 7).

In addition to these results obtained during the sleep–wake cycle, prominent characteristics of LC neurons not previously reported were observed and analyzed. LC neurons were found to exhibit marked fluctuations in discharge rate time-locked to mild, painless sensory stimulations and to EEG and behavioral arousal changes. Such observations were possible because, unlike the situation in previous studies, the experimental subjects were awake during much of the recording, were minimally restrained, were presented with a large variety of sensory stimuli, and were not excessively habituated to the recording situation.

Although rat and monkey LC neurons were similar in many respects (e.g., range of discharge rates, unambiguous but variable sensory responses, and fluctuations in discharge rate closely linked to EEG changes) there were also notable differences. Rat neurons responded more vigorously to simple sensory stimuli such as tones, and they continued to respond over more stimulus repetitions. In contrast, monkey neurons responded most consistently to more complex novel stimuli and exhibited greater variability in discharge rate during the waking state, possibly because the monkeys' level of attentiveness was more labile.

While these data demonstrate that LC-NE neuron activity varies as a function of sensory stimulation as well as arousal level, they do not indicate which of these two factors is the more potent influence on these cells. These neurons appear to be similar to certain reticular neurons in combining properties of sensory responsiveness with those of state-relatedness. Their activity, however, seems less state determined than that recently reported for serotonin-containing neurons of the cat raphe region (22).

The present data provide reasonable predictions about the specific behavioral and environmental conditions that produce synaptic release of NE within the waking state in behaviorally active intact animals. The present results are similar to those from previous studies of LC sensory responsivity in anesthetized or decerebrated preparations in demonstrating short latency biphasic responses to stimuli (12–14). However, the present results indicate that LC activity in anesthetized or decerebrated animals is not characteristic of LC activity in freely behaving intact animals: Whereas LC neurons responded almost exclusively to noxious stimuli in these previous studies, the present data demonstrate that in awake behaviorally active animals LC neurons respond vigorously to mild, nonnoxious, physiologically relevant stimuli as well. This broader spectrum of sensory responsivity implies that LC subserves a much broader role in sensory information processing than participation in nociception.

Thus, these data provide a sharpened perspective on the possible function(s) of LC. Because many of the efferent projections and postsynaptic effects of LC neurons are known, the data presented here permit specific predictions about the role of LC in controlling physiological activity throughout major brain regions. One hypothesis that has received experimental support is that LC-NE neuron activity biases target neurons to respond with enhanced signal-to-noise ratios to subsequent sensory stimuli (23–26).

G.A.-J. was principal investigator for the rat studies reported here; S.L.F. was principal investigator for the monkey studies. A. Schwartz

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1. Ramm, P. (1979) *Behav. Neural Biol.* **25,** 415–448.
2. Clark, T. K. (1979) *Behav. Neural Biol.* **25,** 271–300.
3. Amaral, D. G. & Sinnamon, H. M. (1977) *Prog. Neurobiol.* **9,** 147–196.
4. Steriade, M. & Hobson, J. A. (1976) *Prog. Neurobiol.* **6,** 155–376.
5. Chu, N.-S. & Bloom, F. E. (1974) *J. Neurobiol.* **5,** 527–544.
6. Hobson, J. A., McCarley, R. W. & Wyzinski, P. W. (1975) *Science* **189,** 55–58.
7. Steriade, M. & Hobson, J. A. (1976) *Prog. Neurobiol.* **6,** 328–334.
8. Chu, N.-S. & Bloom, F. E. (1974) *Brain Res.* **66,** 1–21.
9. Jones, B. E. & Moore, R. Y. (1974) *J. Comp. Neurol.* **157,** 43–52.
10. Swanson, L. W. (1976) *Brain Res.* **110,** 39–56.
11. Hubbard, J. E. & DiCarlo, V. (1973) *J. Comp. Neurol.* **147,** 553–566.
12. Cedarbaum, J. M. & Aghajanian, G. K. (1978) *Life Sci.* **23,** 1383–1392.
13. Korf, J., Bunney, B. S. & Aghajanian, G. K. (1974) *Eur. J. Pharmacol.* **25,** 165–169.
14. Takigawa, M. & Mogenson, G. J. (1977) *Brain Res.* **135,** 217–230.
15. Lader, M. H. (1974) *Int. Pharmacopsychiat.* **9,** 125–137.
16. Gray, J. A., McNaughton, N., James, D. T. D. & Kelly, P. H. (1975) *Nature (London)* **258,** 424–425.
17. Redmond, D. E., Jr. & Huang, Y. H. (1979) *Life Sci.* **25,** 2149–2162.
18. German, D. C. & Fetz, E. E. (1976) *Brain Res.* **109,** 497–514.
19. Foote, S. & Bloom, F. (1979) in *Catecholamines: Basic and Clinical Frontiers,* eds. Usdin, E., Kopin, I. & Barchas, J. (Pergamon, Elmsford, NY), pp. 625–627.
20. Jones, G., Segal, M., Foote, S. & Bloom, F. (1979) in *Catecholamines: Basic and Clinical Frontiers,* eds. Usdin, E., Kopin, I. & Barchas, J. (Pergamon, Elmsford, NY), pp. 643–645.
21. Siegel, S. (1956) *Nonparametric Statistics* (McGraw-Hill, New York).
22. Trulson, M. E. & Jacobs, B. L. (1979) *Brain Res.* **163,** 135–150.
23. Foote, S., Freedman, R. & Oliver, A. (1975) *Brain Res.* **86,** 229–242.
24. Freedman, R., Hoffer, B. J., Woodward, D. J. & Puro, D. (1977) *Exp. Neurol.* **55,** 269–288.
25. Segal, M. & Bloom, F. E. (1976) *Brain Res.* **107,** 513–525.
26. Mason, S. T. & Iversen, S. D. (1979) *Brain Res. Rev.* **1,** 107–137.